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Immunology

Positive selection of major histocompatibility complex-restricted suppressor T cells bearing the predominant idiotype in the immune response to lysozyme

(major histocompatibility complex-restricted helper T cells/suppressor-inducer T cell/*Ir* gene control/antigen-pulsed macrophage monolayers)

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ABSTRACT The lysozyme system provides an excellent model for studying the role of multiple major histocompatibility complex (MHC) genes in the induction and regulation of *Ir*-gene controlled immune responses. Immunization of *H-2*^{b} mice leads to concomitant activation of helper and suppressor activities by different epitopes on hen egg-white lysozyme (HEL) and thus phenotypic unresponsiveness to native HEL. HEL-specific suppressor T cells in C57BL/10 nonresponder mice show MHC restriction, because their enrichment on antigen-pulsed macrophage monolayers requires syngeneic macrophages as well as HEL. The expression of the selected suppressor function requires interaction between the restricted suppressor precursor cell and an HEL-triggered, suppressor-inducer T cell. The MHC-restricted suppressor precursors bear the predominant idiotype found on anti-HEL antibodies, whereas MHC-restricted helpers do not.

Thymus-derived (T) lymphocytes play a decisive role in the regulation of antibody responses, displaying helper, amplifier, and suppressor activities. Since its proposal for a guinea pig model (1–3), the concept that most T cell functions are associated with, and regulated by, the major histocompatibility complex (MHC) has become firmly established for the mouse model (4–15) as well. Although the mechanism of induction and expression of suppressor T cells still requires clarification, regulation of suppression by MHC genes is most striking in strains of mice genetically nonresponsive to certain random polypeptide antigens (16, 17). Nevertheless, it has not been conclusively established that the induction of suppressor activity is MHC-restricted, leaving suppressor systems an exception to the usual rules governing T-cell behavior (18).

Studies in our laboratory have focused on mechanism(s) controlling the immune response to hen egg-white lysozyme (HEL). We have defined a highly specific and potent suppressor cell involved in the regulation of the antibody and T-proliferative responses to HEL (19, 20). The unique features of the response in *H-2*^{b} mice are: (*i*) suppressor cells are specific for a single determinant area on the molecule near the NH_2 terminus and conceal the expression of a potent helper T cell response directed against epitopes on the rest of the molecule (21, 22); (*ii*) the genetic nonresponsiveness can be eluded such that soon after immunization in the hind footpad, mice develop IgG plaque-forming cells (PFC) and serum antibody responses, as well as helper and proliferative T cell function in the draining popliteal lymph nodes (P-LN) (19); (*iii*) in the spleen and the long-term primed lymph node, suppression is dominant (23) and requires the interaction of at least two synergizing T cells.

In this communication we will describe a series of experiments that explore the regulation of help *and* suppression by the H-2 complex in the *in vitro* response to HEL. Evidence will be presented that both helper and suppressor precursors are MHC-restricted and are amenable to positive selection on syngeneic HEL-pulsed macrophage (Mϕ) monolayers. Furthermore, we explore the idiotype expressed by these T cell types, insofar as a predominant idiotype (IdX-HEL) characterizes most serum anti-HEL (24) and suppressor T cells (25). It will be shown that the positively selected MHC-restricted suppressor T cells bear IdX-HEL, whereas MHC-restricted helper T cells do not.

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MATERIALS AND METHODS

Mice. Eight- to 12-week-old female C57BL/6J (B6), C57BL/10 (B10), B10.D2, (B10 × B10.D2) F1, and (B6 × DBA/2) F1 strains of mice were purchased from the Jackson Laboratories.

Antigens. HEL was obtained from Societa Prodotti Antibiotici, Milan, Italy, and further purified to homogeneity by ion-exchange chromatography in 0.15 M phosphate buffer, pH 7.18 on Bio-Rex 70 (Bio-Rad). Erythrocytes from one individual goat (GRBC) or burro (BRBC) were purchased from Colorado Serum, Denver, CO. HEL-BRBC were prepared at pH 7.2 by a carbodiimide method (26, 27) as an *in vitro* immunogen.

Cell Cultures. Mouse spleen and lymph node cells were cultured by the method of Mishell and Dutton (28) with some modifications. Briefly, 4–5 × 10^6 cells were cultured in 0.5 ml of Eagle's high amino acid culture medium (29) in Linbro culture plates (FB-16-24 TC, Flow Laboratories, Inglewood, CA). Cultures were rocked at 37°C in 2% CO_2 in air for 4 days, without supplemental feeding, and assayed for direct PFC (see *PFC Assay* below).

Primed Cell Populations. *Primed T-cell-enriched populations.* HEL-primed spleen cells were obtained from mice immunized intraperitoneally with 100 μg of HEL in complete Freund's adjuvant (CFA) (Difco). HEL-primed P-LN were obtained from mice immunized in each hind footpad with 25 μg of lysozyme in 25 μl of saline emulsified with an equal volume of CFA. All primed cells used as a source of helper T cells were fractionated on nylon wool columns before addition to culture,

Abbreviations: B6, C57BL/6 mouse; B10, C57BL/10 mouse; BRBC, burro erythrocytes; CFA, complete Freund's adjuvant; GRBC, goat erythrocytes; HEL, hen egg-white lysozyme; IdX-HEL, predominant idiotype characteristic of most serum anti-HEL; αIdX-HEL, anti-idiotype serum directed against IdX-HEL in secondary anti-HEL serum; MHC, major histocompatibility complex; Mϕ, macrophage(s); PFC, plaque-forming cell(s); P-LN, popliteal lymph node(s); Tsi, T suppressor-inducer cell.

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using the method of Julius et al. (30). We have found negligible amounts of helper activity in unprimed or CFA-stimulated spleen or node cells.

B cells and macrophages. Normal unprimed spleen cells were used as a source of B cells and macrophages after elimination of T cells by treatment with anti-T cell serum plus complement (31). Cultures contained 4 × 10^6 anti-T-treated spleen cells per culture well.

PFC Assay. Three identical cultures were pooled and assayed for anti-HEL PFC by a slide modification of the Jerne hemolytic plaque assay (32), in which erythrocyte-absorbed complement (final concentration 2.5%, vol/vol) and the indicator erythrocytes (HEL-GRBC) were added to the agarose before plating.

Preparation of Mϕ Monolayers. Mϕ monolayers were prepared and utilized essentially as described by Swierkosz et al. (15). Peritoneal cells from thioglycollate-induced exudates, initiated 5 days before culture, were incubated in Linbro FB16-24 TC culture plates at a concentration of 1 × 10^6 cells per well at 37°C in an atmosphere of 10% CO_2/90% air. After 4–5 hr, the nonadherent cells were removed by repeated pipetting followed by three washes with warm 5% fetal calf serum/balanced salt solution. The monolayer cultures were then incubated for 18–20 hr at 37°C with or without 100 μg of HEL per well (1 ml) in complete medium.

After this antigen "pulse," the monolayers were washed three times with 5% fetal calf serum/balanced salt solution to remove free HEL and reincubated in complete medium for an additional 4 hr on a rocking platform to allow for additional release of antigen. At this time the Mϕ were washed before the addition of primed T cells to the monolayer.

Fractionation of Helper T Cells on Mϕ Monolayers. The recovered T cells eluted from nylon wool columns were resuspended in RPMI complete medium. Individual wells containing Mϕ monolayers received 5 × 10^6 primed T cells in 0.7 ml. The Mϕ/T cell mixtures were then maintained at 37°C with rocking for 20–22 hr.

After incubation, the cultures were swirled gently and unbound lymphocytes were harvested and used as the nonadherent T cell fraction. The cells removed during two additional gentle washes of the monolayers were discarded. Finally, those T cells strongly adherent to the Mϕ monolayer (adherent T cell fraction) were recovered by repeated pipetting of the cultures with 1.0 ml of 5% fetal calf serum/balanced salt solution. Both adherent and nonadherent fractions were further incubated on small (2.0-ml) Sephadex G-10 columns to remove contaminating Mϕ before use in culture.

Anti-HEL Idiotype Antisera. Production of monoclonal anti-HEL antibody and anti-idiotypic antiserum against the monoclonal antibody will be described in detail elsewhere. Briefly, hybridoma cell lines producing monoclonal anti-HEL antibody, such as AG5, were constructed by fusing lymph node cells from HEL-primed B10.A mice with P3×63Ag8 myeloma cells in polyethylene glycol as described by Gefter et al. (33). Cultures whose supernates showed anti-Ig-facilitated agglutination of HEL coupled to sheep erythrocytes were cloned by limiting dilution and grown as ascites in (B10.A × BALB/c) F1 mice. The ascites fluids were pooled and the hybridoma product was purified by passage over an Affigel 10 immunoadsorbant (Bio-Rad) coupled with HEL.

Anti-idiotypic antiserum was raised in guinea pigs by primary immunization and boost, followed by bleeding at weekly intervals. The pooled sera were passed over an HEL immunoadsorbant. Anti-isotypic and anti-allotypic specificities were then removed by several passages over immunoadsorbant columns coupled with the Ig fraction of a (B10.A × BALB/c) F1 ascites fluid that had been induced with P3×63Ag8 myeloma cells. The activity and specificity of the antiserum were assessed by inhibition of idiotype–anti-idiotype binding in a solid-phase radioimmunoassay (34), adsorption of isoelectrofocusing patterns, and inhibition of HEL binding in a Farr assay (24). The anti-idiotypic antiserum recognized over 90% of the anti-HEL antibody found in immune B10.A sera but did not show detectable reaction with normal B10.A IgG or B10.A anti-human lysozyme antibody.

RESULTS

Associative Recognition of HEL and MHC Gene Products by B10.D2 Lymph Node Helper T Cells. To fulfill the criteria for enrichment of antigen-specific H-2-restricted T helper cells, (B10 × B10.D2) F1 splenic T cells were fractionated on HEL-pulsed syngeneic or allogeneic Mϕ. The results are presented in Table 1. We could demonstrate a 3-fold enrichment in the relative helper activity of T cells adherent to syngeneic macrophage monolayers, compared to unfractionated T cells, in contrast to a reduction to 1/10th in the population adherent to HEL-allogeneic Mϕ. The helper activity recovered in the nonadherent fraction of RNase-syngeneic Mϕ or HEL-allogeneic Mϕ was not significantly different from that of the unfractionated T cells, while the "enriched" fraction in these cases was noticeably depleted. Thus it is clear that HEL-primed splenic helper T cells, in accord with the activity of splenic helper cells studied by Swierkosz et al. (15), recognize both antigen and MHC gene products.

It has been our experience that HEL-primed lymph nodes are a rich source of antigen-specific helper and proliferating T cells, even in nonresponder strains (23, 35). Thus, we performed a similar selection to test for MHC-restricted helper function in responder B10.D2 and nonresponder B10 lymph node cells. The results are presented in Fig. 1. Both B10.D2 and B10 mice were immunized with 50 μg of HEL in CFA and their nylon wool-enriched P-LN T cells were fractionated on HEL Mϕ for comparison of helper activity. When B10.D2 P-LN T cells were incubated on syngeneic H-2^d or allogeneic H-2^b HEL-pulsed Mϕ monolayers and tested in culture with H-2^d B cells, the adherent T cell fraction possessed 9 times the helper activity of the nonadherent fraction, and 21 times greater activity than the adherent fraction from HEL-pulsed allogeneic Mϕ.

Table 1. Specificity of enrichment of HEL-primed helper F1 cells for antigen and MHC

	HEL-primed P-LN cells*			HEL-specific
Exp.	Unfractionated	Adherent to	Nonadherent to	helper activity†
1	Unfractionated	—	—	1115 ± 75
2‡	—	F1-Mϕ-HEL	—	3125 ± 187
		—	F1-Mϕ-HEL	505 ± 120
3§	—	F1-Mϕ-RNase	—	50 ± 3
		—	F1-Mϕ-RNase	1129 ± 83
4‡	—	B10.A-Mϕ-HEL	—	125 ± 17
		—	B10.A-Mϕ-HEL	800 ± 62

* (B10 × B10.D2) F1 mice were immunized with 25 μg of HEL in CFA per hind footpad 10 days before selection. The protocol for selection is described in detail in the text.

† HEL-specific PFC per 10^6 added T cells per culture well ± SEM.

‡ (B10 × B10.D2) F1, or B10.A Mϕ monolayers were pulsed with 100 μg of HEL per ml per culture well.

§ (B10 × B10.D2) F1 Mϕ monolayers were pulsed with RNase, an irrelevant antigen.

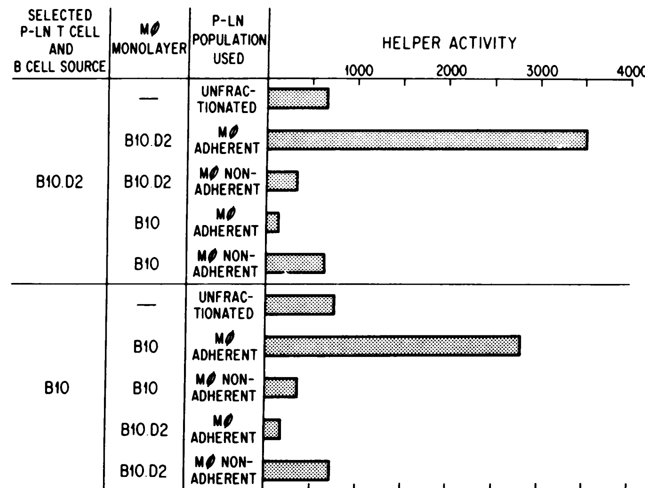

FIG. 1. MHC restriction in helper cell induction. B10 and B10.D2 HEL-immune P-LN T cells were selected for MHC-restricted helper activity. The helper activity of both Mϕ-adherent and nonadherent fractions of lymph node T cells on syngeneic or allogeneic Mϕ was measured in culture with 4×10^6 syngeneic B cells and HEL-BRBC as antigen. Helper activity refers to the number of HEL-specific PFC per 10^6 T cells added to the culture, determined by T cell titrations.

Predictably, helper cells from a peripheral lymph node exhibit the same requirements in their recognition of antigen as do splenic helper cells; that is, associative recognition of antigen and MHC gene products is necessary for expression of function. Next, B10 P-LN T cells were selected on either B10 or B10.D2 HEL-pulsed Mϕ. The lower half of Fig. 1 shows that only syngeneic Mϕ would support enrichment of B10 helpers and affirms the conclusions drawn previously (36) that in the P-LN the induction of HEL-specific helper cells in B10 nonresponders is governed by MHC genes.

Associative Recognition of Antigen and MHC Genes by Suppressor Precursors. Because of the unique functional contrast between P-LN T cells and splenic T cells in recently immunized B10 mice (only the splenic population containing a suppressor precursor cell to HEL), this system seemed particularly suitable for establishing the role of MHC genes in the induction of suppressor precursors. Selection of HEL-specific, B10 splenic T cells was attempted on B10, B10.D2, B10.A, or B10.BR HEL-pulsed Mϕ. Elsewhere we have shown that a suppressor precursor cell bearing the predominant idiotype in the HEL system needs a T-suppressor-inducer (Tsi) cell in order to be activated (23). This $I\text{-}J^+$ Tsi can be found in the P-LN, 10 days after footpad priming with HEL, along with antigen-specific, $I\text{-}J^-$ T helper cells. Therefore, selection of suppressor cells on the Mϕ monolayers was assessed by adding a small number of selected splenic cells (2%) to the HEL-primed P-LN helper/inducer population. The resultant helper activity of the T cell mixes was compared to the activity of 100% P-LN T cells.

As shown in Fig. 2, suppression of P-LN helper activity was quite dramatic. Suppressors were not adherent to Mϕ monolayers unless they were antigen-pulsed (data not shown). The prominent feature of the specificity of this suppressor population is its preference for B10 MHC in association with the antigen. The pattern emerging from the *in vitro* selection of primed T cells suggests that HEL-primed T cell helpers and suppressors can each recognize epitopes on the antigen in the context of *H-2* gene products and can be enriched on macrophage monolayers.

Idiotypic Phenotype of Helper Cells Recognizing HEL in Association with MHC Gene Products. We have previously

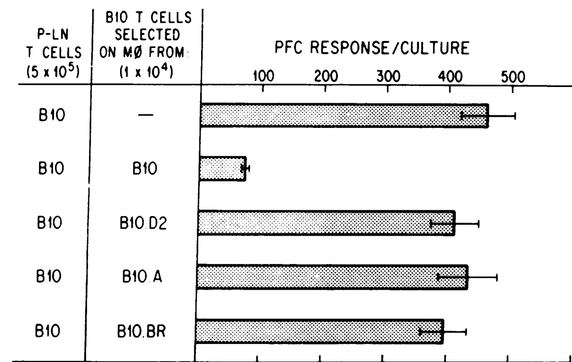

FIG. 2. MHC restriction in suppressor cell induction. In this representative experiment, the selected cells were derived from nylon wool-purified, HEL-immune B10 spleen cells (known to contain suppressors) adhering to HEL-pulsed syngeneic or allogeneic Mϕ monolayers. The test cultures contained 5×10^5 HEL-primed B10 P-LN T cells (helper target and suppressor-inducing populations), 1×10^4 Mϕ-selected T cells (suppressors), and 4×10^6 T-cell-depleted normal B10 spleen cells (B/Mϕ population). The day 4 anti-HEL PFC response per 5×10^5 T cells per culture is shown.

shown that a reagent directed against the predominant idiotype on anti-HEL antibodies (αIdX-HEL$_{43}$), prepared by immunizing guinea pigs with murine affinity-purified anti-HEL serum antibody, obliterated the activity of splenic suppressor precursors in an *in vitro* assay (25). To establish the idiotypic phenotype of the helpers and suppressors in this system, we examined the ability of a similar anti-idiotype reagent raised against the hybridoma AG5 (αIdX-HEL$_{AG5}$) described in *Materials and Methods*, to block the specific adherence of B10 cells recognizing HEL in conjunction with MHC gene products. Nylon wool-enriched P-LN T cells were incubated with αIdX-HEL$_{AG5}$ (without complement) before attempted selection on HEL-pulsed macrophages. Subsequently, Mϕ adherent and nonadherent cell fractions were cultured with B cells and antigen. As depicted in Fig. 3, αIdX-HEL$_{AG5}$ did not interfere with helper T cell recognition of antigen and MHC gene products, because there is no perceptible difference in the percent enrichment of helper activity between the anti-idiotype-treated and untreated T cells. This is consistent with our previous findings using αIdX-HEL$_{43}$, with which no effect on helper activity could be demonstrated at a concentration at which suppressor precursors were completely eliminated. We would conclude

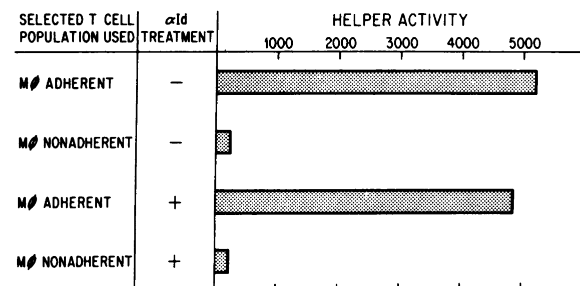

FIG. 3. αIdX-HEL$_{AG5}$ does not prevent antigen recognition by P-LN helper T cells. HEL-immune nylon-enriched B10 T cells were selected for antigen/MHC recognition on HEL-pulsed B10 macrophages. One sample of T cells was exposed at 1:100 dilution of anti-idiotype without complement, for 45 min at 0°C. After centrifugation and resuspension in L-15 medium, positive selection was attempted. Both Mϕ adherent and nonadherent fractions were tested for their helper activity in culture with normal B10 B/Mϕ and HEL-BRBC. Helper activity is defined as in the legend to Fig. 1.

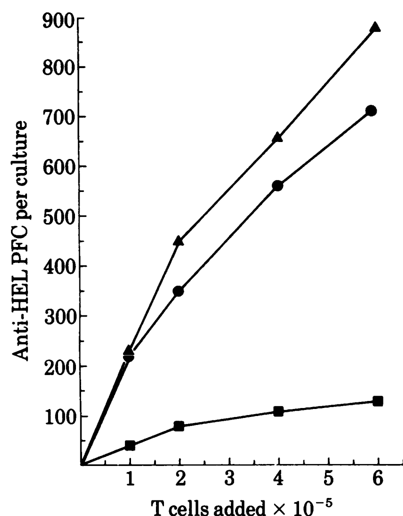

FIG. 4. αIdX-HEL$_{AG5}$ does prevent antigen recognition by splenic suppressor T cells. HEL-immune nylon-enriched B10 T cells were selected on B10 HEL Mϕ. Some of the T cells were exposed to anti-idiotype without complement before selection, as in the legend to Fig. 3. Both Mϕ adherent and nonadherent fractions were tested for suppressor activity by mixing a ratio of 2% splenic T cells and 98% P-LN T cells and titrating the mixed population into cultures of 4×10^6 B/Mϕ plus antigen. ●, Titration of helper activity in P-LN T cells; ■, the same P-LN helpers plus Mϕ adherent suppressors; ▲, the same P-LN helpers plus Mϕ adherent suppressors pretreated with αIdX-HEL$_{AG5}$.

from this experiment, as well as others performed by different analytical methods, that B10 HEL helpers do not display the predominant idiotype found on anti-HEL antibodies.

Idiotype Phenotype of Suppressor Precursors Recognizing HEL in Association with MHC Gene Products. Harvey *et al.* (25) have shown that the suppressor precursor that is primed by HEL after intraperitoneal injection bears the major cross-reactive idiotype of anti-HEL, and the experiment depicted in Fig. 2 indicates that suppressor precursors recognize antigen together with MHC gene products. Yet it is not clear whether the suppressor precursor (idiotype-positive) is itself restricted, or whether two independent suppressor subsets are involved. Therefore, HEL-immune splenic T cells were selected on HEL-pulsed Mϕ monolayers after exposure to the αIdX-HEL$_{AG5}$ reagent. The adherent cells were assayed for suppressor function by admixture with B10 P-LN cells as a source of helpers and suppressor inducers. The titration of the HEL-primed P-LN helpers shown in Fig. 4 demonstrates the strength of P-LN T cell helper activity 10 days after *in vitro* priming (1360 PFC per culture). After admixture with selected adherent splenic T cells, the helper activity was reduced to 20% (246 PFC per culture) even at a ratio of 2% spleen to 98% P-LN. Previous titrations have shown that this suppressor precursor activity is still potent at a 0.3% ratio of suppressors to helpers.

The critical result is that the anti-idiotype blocked the suppressor precursor from Mϕ adherence. The helper activity was not at all compromised by admixture with selected Mϕ-adherent cells. In fact, the activity was slightly enriched (1640 PFC per culture). In any event, the suppressive population could not be retrieved from the Mϕ monolayers: the nonadherent population was not examined, and presumably would have contained th anti-idiotype-blocked cells. We conclude that anti-idiotype antiserum effectively blocked the suppressor precursors from recognition of antigen associated with MHC gene products. Accordingly, the suppressor precursor cell whose expression is dependent upon an inducer cell bears the predominant HEL idiotype and is triggered by antigen in the context of MHC gene products.

DISCUSSION

The concept of associative antigen recognition by T cell subpopulations has been shown previously to apply to the induction of cytotoxic and helper cells. In this report we have presented evidence for MHC restriction of both helper and suppressor T cell induction in the lysozyme system.

Presence of MHC-Restricted Helper Cells in Both Responder and Nonresponder Mice. We have shown that HEL-specific T helper activity is present in responder (B10 × B10.D2) F1 splenic T cells and that this activity becomes substantially enriched by fractionation on HEL-pulsed Mϕ monolayers. The specific enrichment requires both antigen and Mϕ, thus linking the recognition of antigen and MHC gene products. The induction of HEL-specific T helper cells in a nonresponder strain (B10), accomplished by immunization in the hind footpad, complies with the rules of recognition so well documented by others (5, 7–9, 13–15, 37). The enrichment for antigen-specific cells, in this case, can occur only with syngeneic monolayers, and the adherent T cells provide help only when the B/Mϕ population is syngeneic (36). Incubation of (responder × nonresponder) F1-primed splenic T cells on either responder or nonresponder Mϕ leads to the selection of T cells that recognize the identical gene products on responder or nonresponder B/Mϕ cells. Again, only the B/Mϕ cell source syngeneic to the "selecting" Mϕ will fulfill the requirements for T–B collaboration.

Positive Selection of Either Suppressors or Helpers on HEL-Pulsed Mϕ Monolayers. The results in Figs. 1 and 2 demonstrate decisively that the "nonresponder" B10 mice are perfectly capable of developing MHC-restricted help in the 10-day P-LN and MHC-restricted suppression in the 10-day spleen [presumably directed against the N-C portion of HEL (21, 22), which is a peptide consisting of amino acids 1–17 and 120–129, with a disulfide bond between cysteine-6 and cysteine-127]. Only the splenic T cells adherent to nonresponder macrophages showed any suppressor activity; nonadherent cells had neither suppressor nor helper activity (data not shown).

Our previous experiments indicated that suppression in the lysozyme system required the synergy of two primed T cells, one of which, an inducer of suppression, Tsi, is resident in 10-day primed, nonresponder lymph nodes, concomitantly with helper cells (23). Our results are in full agreement with those of Eardley, Gershon, Cantor, and coworkers (38, 39) that, without the Tsi, the suppressor precursor activity remains quiescent.

The results obtained in this study have been extended to explore the regulation of HEL-specific, MHC-restricted helper cells in the (responder × nonresponder) F1. In preliminary studies published elsewhere (36), we showed that only nonresponder Mϕ could be used for positive selection of a population of suppressor precursors from F1 spleen cells, demonstrating that MHC-restricted suppressors are detectable only for the *H-2*^{b} haplotype. On the other hand, helper T cells were selected on the appropriate Mϕ for either parental haplotype. Concomitant positive selection of helpers and suppressor-precursors but not suppressor-inducers, in this antigen-pulsed Mϕ system, permits the deduction that the suppressor-inducer cell is not antigen-specific. These results, as well as the results reported in this communication, emphasize the requirement for coupled MHC and antigen recognition by suppressor cell precursors.

With respect to the attempted positive selections of suppressor or helper precursors on allogeneic macrophages, we feel

that it is highly improbable that negative or positive allogeneic effects arising from contaminant Mϕ are responsible for our results. The selected cells were filtered through Sephadex G-10 columns before culture and appeared to be 99% lymphocytic. Secondly, *both* "helper selections" and "suppressor selections" attempted on allogeneic macrophages were failures. These contrasting effects of the putative contaminants is not consistent with predictions involving allogeneic effects.

The precise MHC gene(s) involved in the restriction remain to be identified. Recent studies in several laboratories (40–42) support the notion that the K/D type I proteins could be critical in suppressor cell regulation and induction.

Characterization of the Idiotypic Markers on Suppressor and Helper Cells. Because of the extensive work performed in this laboratory concerning the specificity of help and suppression in the lysozyme system, we have a reasonable expectation of defining the specificity of the receptors on the cell types involved in the production of helper and suppressor T cells. In proliferation assays in which the activity measured has the same specificity pattern as the helper T cell repertoire, B10 T cells seem primarily directed against certain determinants on the HEL molecule within the L_{II} peptide (the major cyanogen bromide peptide, amino acids 13–105) (21). Suppressor cells, on the other hand, are directed against a determinant at the NH_2 terminus, contained within the N-C peptide. The N-C peptide itself can induce suppression, and the suppressor precursor cell bears the major idiotype found on the overwhelming majority of antibody molecules produced in the anti-HEL response of both responders and nonresponders. Thus, it was reasonable to expect that the MHC-restricted, HEL-pulsed Mϕ adherent suppressor cell would also be the idiotype-bearing suppressor precursor. The blocking experiments demonstrate that an anti-idiotypic reagent discriminated between idiotype-positive and idiotype-negative cell types and thus prevented the ultimate recognition/adherence to antigen and MHC structures on the Mϕ surface via antigen receptors. We cannot rule out the possibility that the MHC-restricted nature of the suppressor precursor cell is derived from an interaction between an MHC-restricted cell and a non-MHC-restricted, antigen-specific suppressor precursor during the fractionation procedure. Whereas the suppressor precursors were prevented from being positively selected by pretreatment with the anti-idiotypic serum, this serum completely failed to interfere with the positive selection of the helper population (presumably L_{II}-specific).

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